

EcoPURE BYF Genomic DNA Kit

Troubleshooting Guide

50 rxns

Cat No: E1085

Use this guide for common problems encountered during genomic DNA purification from bacteria, yeast, and fungi. The recommendations below follow the sample limits, lysis, enzymatic treatment, binding, wash, centrifugation, and elution conditions specified in the product datasheet.

Before You Start - Quick Checks

1. Before first use, add 8.8 mL absolute ethanol to EcoPURE Wash Buffer 1 and 32 mL absolute ethanol to EcoPURE Wash Buffer 2.
2. Reconstitute lyophilized Proteinase K in 1.1 mL Proteinase K Storage Buffer and RNase A in 1.1 mL RNase A Reconstitution Buffer.
3. Standard inputs are: 1 mL overnight bacterial culture; up to 10⁸ yeast cells; or 100-150 mg wet fungal mycelia/spores or pellets from 0.5-3 mL fungal culture.
4. For most gram-positive bacteria, use lysozyme (not provided). For yeast, use 10 µL lyticase (0.5 mg/mL; not provided) for 30 minutes at 37°C.
5. Use 20 µL EcoPURE Proteinase K and the sample-specific 55°C incubation. For bacteria/yeast, 10 minutes is standard and may be extended to 30 minutes; fungi use 30 minutes.
6. For fungal samples, clarify the lysate for 1 minute at maximum speed and transfer only the supernatant before RNase A and binding steps.
7. Complete the required wash sequence and final 200 µL Wash Buffer 2 / 2 minute maximum-speed spin to remove residual wash buffer.
8. Elute with 30-50 µL EcoPURE Elution Buffer applied to the membrane center; incubate 5 minutes at room temperature before centrifugation.

Application	Problem	Most likely cause	Recommended action
General	Low or no genomic DNA yield	Incomplete cell disruption or lysis; sample input above the stated protocol capacity without proportional reagent scaling; Proteinase K step incomplete; Binding Buffer omitted; or incomplete sample transfer to the column.	Follow the sample-specific lysis procedure and stated input limit. Resuspend or disrupt the sample completely, perform the Proteinase K incubation as specified, add the required Binding Buffer, transfer the full sample to the EcoPURE Column, and centrifuge at the stated maximum speed.
General	Variable DNA yield between samples	Differences in biomass, pellet resuspension, lysis efficiency, incubation time, reagent scaling, wash steps, or elution conditions.	Standardize sample input and handling. Keep reagent volumes, incubation temperatures/times, centrifugation conditions, wash sequence, and the 30-50 µL elution volume consistent between comparable samples.
General	RNA contamination in purified DNA	RNase A treatment was omitted or not performed according to the sample-specific protocol.	Reconstitute RNase A as instructed before use. For bacteria, the protocol allows an optional 20 µL RNase A treatment for 3 minutes at room temperature. For yeast and fungi, use the specified 20 µL RNase A treatment and 3 minute room-temperature incubation.
General	Poor downstream PCR/qPCR or enzymatic performance	Residual wash buffer or ethanol remained on the membrane, or wash reagents were not prepared correctly before use.	Prepare Wash Buffer 1 with 8.8 mL absolute ethanol and Wash Buffer 2 with 32 mL absolute ethanol. Complete the sample-specific wash steps and the final 200 µL Wash Buffer 2 / 2 minute maximum-speed spin before elution.
General	Low DNA concentration in eluate	Elution buffer was not applied to the membrane center, the 5 minute incubation was shortened, or an inconsistent elution volume was used.	Apply 30-50 µL EcoPURE Elution Buffer directly to the center of the membrane, incubate the column for 5 minutes at room temperature, then centrifuge for 30 seconds at maximum speed.
General	Column flow is slow / liquid remains on the column	Cell clumps, incompletely disrupted material, fungal debris, or an oversized sample was loaded onto the membrane.	Ensure complete pellet resuspension or sample disruption before loading. For fungi, perform the 1 minute maximum-speed clarification spin and transfer only the supernatant. Keep sample input within the stated protocol range or scale reagents proportionally as directed.
General	Proteinase K treatment appears ineffective	Lyophilized Proteinase K was not reconstituted correctly, the 55°C incubation was shortened, or incubation time was insufficient for the sample.	Reconstitute Proteinase K in 1.1 mL Proteinase K Storage Buffer. Use 20 µL per standard preparation and incubate at 55°C for the specified time. For bacterial and yeast samples, extending the 10 minute incubation to 30 minutes may increase yield; fungi use 30 minutes as the standard incubation.
Bacteria	Bacterial pellet does not resuspend completely	Pellet was not dispersed fully in Resuspension Buffer, leaving visible cell clumps before lysis.	Resuspend the pellet in 200 µL EcoPURE Resuspension Buffer by vortexing or pipetting up and down until no cell clumps remain, then add 200 µL EcoPURE Lysis Buffer.
Bacteria	Low yield from gram-positive bacteria	The thick peptidoglycan cell wall was not sufficiently disrupted.	For most gram-positive bacteria, use the kit together with lysozyme (not provided) as indicated in the datasheet to improve cell-wall lysis before continuing with the standard workflow.
Bacteria	Low yield from more than 1 mL culture	Culture volume exceeded the standard 1 mL input while reagent volumes remained unchanged.	The standard procedure is for 1 mL overnight bacterial culture. If a higher culture volume is required, scale the amounts of reagents used in the entire protocol proportionally.

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Troubleshooting Guide (continued)

Application	Problem	Most likely cause	Recommended action
Bacteria	Yield remains low after standard lysis	The 10 minute Proteinase K incubation was insufficient for the bacterial sample.	After adding 20 µL EcoPURE Proteinase K, incubate at 55°C for 10 minutes. The datasheet states that extending this incubation to 30 minutes can help increase yield.
Yeast	Low yield from yeast cells	Lyticase treatment was omitted, insufficient, or performed under incorrect conditions.	After resuspending the pellet in 200 µL EcoPURE Resuspension Buffer, add 10 µL lyticase (0.5 mg/mL; not provided) and incubate for 30 minutes at 37°C before adding EcoPURE Lysis Buffer.
Yeast	Yeast pellet remains clumped	The pellet was not completely dispersed before the lyticase and lysis steps.	Resuspend the yeast pellet in 200 µL EcoPURE Resuspension Buffer by vortexing or pipetting until no cell clumps remain, then proceed with the lyticase treatment.
Yeast	Low yield with high yeast-cell input	More than 10 ⁸ cells were processed with the standard reagent volumes.	The standard procedure is suitable for up to 10 ⁸ yeast cells. If more cells are required, scale the amounts of reagents used in the entire protocol proportionally.
Yeast	Residual contaminants after purification	Wash Buffer 1 or Wash Buffer 2 step was omitted, or the final dry-spin step was incomplete.	After column binding, wash sequentially with 400 µL Wash Buffer 1 and 500 µL Wash Buffer 2, centrifuging 30 seconds after each. Then add 200 µL Wash Buffer 2 and centrifuge 2 minutes at maximum speed.
Fungi	Low yield from mycelia, spores, or fungal pellets	Mechanical disruption in liquid nitrogen was incomplete, tissue lysis was insufficient, or the Proteinase K incubation was too short.	Grind the sample in liquid nitrogen to a fine powder, resuspend in 200 µL Resuspension Buffer, then add 200 µL Tissue Lysis Buffer plus 20 µL Proteinase K and incubate for 30 minutes at 55°C. Extending incubation can increase yield.
Fungi	Fungal lysate contains heavy debris / column clogs	Particulate material was carried forward because the clarification step was omitted or the pellet was disturbed.	After the 55°C lysis, centrifuge at maximum speed for 1 minute at room temperature. Transfer the supernatant to a new 1.5 mL tube and leave the pellet/debris behind before RNase A and binding steps.
Fungi	Poor DNA binding to the column	The fungal binding mixture was prepared without the required absolute ethanol or was not mixed well.	After RNase A treatment, add 400 µL EcoPURE Binding Buffer, then add 200 µL absolute ethanol and mix well before loading the sample onto the EcoPURE Column.
Fungi	Low yield with large fungal sample	Sample exceeded 100-150 mg wet mycelia/spores or the equivalent pellet from 0.5-3 mL culture without proportional reagent scaling.	Use the standard input range stated in the datasheet. If a larger fungal sample is required, scale the amounts of reagents used in the entire protocol proportionally.
Fungi	Yield varies during the 55°C fungal lysis	The sample was not mixed during the 30 minute incubation or lysis remained incomplete.	Incubate with Tissue Lysis Buffer and Proteinase K for 30 minutes at 55°C. The datasheet notes that vortexing the sample every 5 minutes during incubation may increase DNA yield.

Still having trouble? Re-check sample input, complete resuspension or mechanical disruption, lysozyme/lyticase use where required, Proteinase K and RNase A preparation, binding conditions, ethanol addition to the wash buffers, the final 2 minute Wash Buffer 2 spin, and the 5 minute elution incubation. If the problem persists, contact EcoTech Biotechnology technical support with the organism/sample amount, reagent volumes, enzyme treatment, incubation conditions, centrifugation steps, and observed DNA yield or downstream issue.